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ARTIFICIAL ADJUSTMENT OF FLOWER PERIOD AND HYBRIDIZING TECHNIQUES OF *AMORPHOPHALUS KONJAC*

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Abstract Under natural conditions, the four - to - five - year - old plants of *A. konjac* bloom in April. By crossing with *A. corrugatus*, normal hybrid seeds were harvested. When konjac corms with flower buds were stored at 6 - 9℃, their flower period was delayed by about 4 months, thus can overlap with the flower period of *A. albus* and make the crosses between the two species possible. Hybrid seeds of reciprocal crosses were obtained. By pre - treating the corms with no flower buds with $100x10^{-6}$ GA_3, male - sterile flowers were induced. When pollinated with pollen of *A. albus* and other species, normal seeds were obtained. Cultivation of the hybrids thus obtained shows that the F1 generation were matroclinous in character. Strains of excellent characters were selected from the hybrid population.

Key words *Amorphophallus konjac*, artificial adjustment, crossbreeding

Konjacs are perennial herbs belonging to *Amorphophallus*, Araceae. Among the world's over 130 species, China possesses 21 (Li and Long, 1998), of which six are cultivated in China. *Amorphophallus konjac* C. Koch is the most widely distributed and cultivated species in China, with its production accounting for more than 80% of China's total yield. However, due to its poor resistance especially to diseases, its quality is in great need of improvement. It is of great importance to improve it through cross - breeding and so that our plentiful konjac resources can be better utilized. At present, cross breeding of konjac has many technical problems which still need to be solved.

CROSSES BETWEEN *AMORPHOPHALLUS KONJAC* AND OTHER SPECIES UNDER NATURAL CONDITIONS

Under natural conditions in 1992 - 1993 when the spring temperature in Chongqing raised to 15℃, the flower buds of *A. konjac* began bursting out and blooming in April and May. This coincides with that of *A. corrugatus* N. W. Brown and *A. paeoniifolius* N. E. Brown. But no normal hybrid seeds were produced in reciprocal crosses. After excision of the stigma of *A. konjac*, reciprocal crosses with *A. corrugatus* yielded normal seeds, while in crosses with *A. paeoniifolius*, no seeds were obtained. This shows that there is interspecific isolation between A. konjac and A. corrugatus. This barrier can be overcome by the stigma excision of the former. The isolation between *A. konjac* and *A. paeoniifolius*, however, cannot be overcome by this method.

ARTIFICIAL ADJUSTMENT OF FLOWER STAGE OF *A. KONJAC*

1. Storage of Corms with Flower Buds

(1) Materials and Methods

To solve the problem of differing flower periods of *A. konjac* and other species, corms with flower buds of *A. konjac* were stored (from March 12) in refrigerator (6 - 9℃) in 1992 before spring temperature raised to 15℃. In the first ten groups were taken out on April 20. After that, every 5 days until June 10. Other flowering corms were stored beginning on February 20, 1993. In the first eight groups were taken out on April 15,

and then every 7 days until June 5.

(2) Results and Analyses

The result of the experiment in 1992 shows that *A. konjac* began blooming from April 22 under indoor cultivation, and continued until May 10. After low – temperature storage, the flower period was from May 5 to June 29, a delay of 50 days. Them can meet the flower period of *A. albus* P. Y. Liu et J. F. Chen, which under natural conditions, blooms from May 29 to June 12.

A similar result was obtained in 1993, in which the flower period was from May 20 to July 2, after storing the corm for 50 days. This overlapped with that of *A. albus* (from June 1 to July 5).

The above results show that storing the flower corms at low temperature can delay the flower period of *A. konjac*, and by taking out in groups, the flowering period can be delayed to as late as July 2. Thus it can result in synchronization of flower periods and create conditions for reciprocal crosses.

2. Induction of Flower Buds from Corms without Flower Buds

(1) Materials and Methods

In March 1992 and 1993, corms with no flower buds (about 500g) were presoaked in 100×10^{-6} gibberellin acid (GA_3) for 12 hours. They were then cultivated indoors and outdoors to examine the flower period, the pollen dispersal, and the pollen structure. The female flowers were artificially pollinated for observation.

(2) Results and Analyses

The results of the two years showed that in early June, inflorescences and leaves were successfully induced. Normal seeds were obtained when pollinated with pollen of either *A. albus* or *A. konjac*. The GA_3 – induced pollen were morphologically normal, but anthers did not open both under natural outdoor conditions and under indoor high temperature conditions. When placed in dry conditions, the pollen became dry and shrinking, and its inner wall became brownish black. The amount of pollen was scarce about one – tenth of the normal. No fertile pollen was found.

These results show that GA_3 – induced pistils of *A. konjac* were fertile and the stamen, sterile. This can solve the problem of differing flower periods of *A. albus* and *A. konjac*. What's unsatisfactory is that GA_3 – indcued inflorescence can only be used as female parent, and it can not cross reciprocally at the same time.

3. The Storage of Pollen

(1) Materials and Methods

Fresh pollen of *A. konjac* was stored under room temperature (22℃) (dry or humid conditions), +5℃ (dry condition) and −18℃ (dry condition). It was taken out every 12h and incubated in a medium containing 0.8% agar, 10% sucrose, 50×10^{-6} boric acid (pH6.0) at 25℃ to measure germination rates.

(2) Results and Analyses

The results showed that the pollen of *A. konjac* was viable for only 3 – 4 days at 22℃, but it was viable for 28 days at −18℃ This shows that the pollen of *A. konjac* is suitable for long – term storage. In addition, *A. konjac* can only be used as male parent, and cannot be crossed reciprocally at the same time.

4. Cross Breeding Techniques

(1) Isolation by Bagging

a. Materials and Methods

For bigger inflorescences of *A. konjac* and *A. corrugatus*, white cloth bags and waterproof paper were used for isolation. For the smaller ones, bigger sized craft – paper envelopes were used. For the male parent, the time of bagging was just before the full opening of inflorescences. The pollen began to disperse in about 24 hours. After collection of the pollen, bags remained for 3 days until the plants growing nearby were pollinated.

For the females, bags were taken off 48 hours after pollination.

b. Results and Analyses

Both cloth and paper bags are very good for isolation. But for a longer periods of isolation, the high temperature and high moisture inside the bags could readily mold of stamen and pistils, and this may also affect the development of seeds.

(2) Artificial Emasculation

a. Materials and Methods

Two ways of emasculation of female parents were used; one was to cut the pistil, and then apply a layer of vaseline to prevent cross pollination; the other was to apply a layer of vaseline directly over the pistil to prevent pollen from dispersal.

b. Results and Analyses

Most of those pistils that were cut were viable, and when crossed can produce seeds. Those that were not cut did disperse pollen from anther which made the male inflorescence black. But even so, the pollen still could not disperse out of the vaseline layer into the air. Evidently these two methods are effective in emasculation. The latter is better in that it does not physically harm the plant.

Table 1. Effects of Chemicals on Emasculation

Material	Chemical	Treatment	Result
A. konjac flower	1000×10^{-6} GA_3	Daubing Pistil	Pollen dispersal, viability 34.2%
A. konjac flower	70% EtOH	Daubing Pistil	Pollen dispersal, viability 15.4%
A. konjac flower	Satur $KMnO_4$	Daubing Pistil	Pollen dispersal, viability 26.4%
A. konjac flower	0.1% $HgCl_2$	Daubing Pistil	Pollen no dispersal
Corm without flower bud	1000×10^{-6} GA_3	Pre – soaking	Pollen no dispersal

(3) Emasculation by Chemicals

a. Materials and Methods

The male inflorescence of *A. konjac* and corms without flower buds, chemicals, methods of treatment see Table 1.

b. Results and Analyses

It can be seen from Table 1 that treatments with 0.1% $HgCl_2$ and 100×10^{-6} GA_3 are more effective in making the pistil sterile and all the others can not cause complete sterile but only reduce the viability of pollen. Probably it is because of either inadequate concentration or inproper treating time or different penetrating ability of chemicals into pistil.

(4) Hybrid Combinations and Techniques

a. Materials and Methods (see Table 2.)

b. Results and Analyses

Table 2 shows that, during 1992 and 1993, *A. konjac* and *A. albus* were able to cross reciprocally. This suggests that they are compatible in crossing. Reciprocal crosses of *A. konjac* and *A. paeoniifolius* produced no seeds. When excising its stigma and then pollinated, the ovary seemed normal during the first 25 days, and then gradually softened and finally died out. This suggests that there exists comparable incompatibility between them. Under natural conditions, crosses between *A. konjac* and *A. corrugatus* had same results. During the 30 days after pollination, the ovary became softening and then rotten, also belonging to embryonic sterility. This suggests that certain incompatibility exists between them. But this can be overcome by

Table 2. Relations Between Hybrid Combinations and Seeds Harvested

Test time	Combination	Treatment	No. of strains hybridized	No. of strains yielding normal seeds
1992	*A. konjac* × *A. albus*	normally pollinated GA_3 – induced female pollinated on excision of stigma	25 20 5	14 5 1
	A. konjac × *A. konjac*	GA_3 – induced female	6	2
	A. albus × *A. konjac*	normally pollinated excised stigma 4% sucrose daubed	2 2 2	1 1 1
	A. konjac × *A. paeoniifolius*	normally pollinated excised stigma	1 1	0 0
	A. paeoniifolius × *A. konjac*	excised stigma	1	0
1993	*A. albus* × *A. konjac*	GA_3 pre – soaked female	5	0
	A. konjac × *A. albus*	GA_3 – induced female	5	3
	A. konjac × *A. corrugatus*	normally pollinated excised stigma	5 17	0 5
	A. corrugatus × *A. konjac*	normally pollinated excised stigma	3 5	0 1

the excision of stigma. Budless corms of *A. konjac*, when presoaked in 100×10^{-6} GA_3, could be pollinated by either *A. konjac* or *A. albus*. Its stamen was sterile and pistil fertile. It is a satisfactory male sterile material.

(5) Morphological Observations of the F_1 Generation

The stem of *A. konjac* was dark – black, and that of *A. albus*, pure green (indigo). Stem color of. the F_1 generation of *A. konjac* × *A. albus* was between that of its parents. Its stripes varied with reciprocal crosses. The stem of F_1's of *A. konjac* × *A. albus* was ink – black on a pink background, whereas that of *A. albus* × *A. konjac* was dark – green on a tender – green background. Leaf stalk of one year old F_1's between *A. corrugatus*, *A. konjac*, and *A. albus* was yellow – green with dark – green stripes. The cross section of their corms, were light – yellow, more like that of *A. corrugatus*. According to our observation and the records of other literature (Liu *et al*, 1986), the rhizomes of *A. konjac* usually begin to develop in plants that are two or more years old. However, in our experiment we observed that one year old F1's plant developed several rhizomes. Further study has to be conducted to ascertain whether it is unique in hybrids. We also found that certain F_1 seeds of *A. konjac* × *A. albus*, *A. konjac* × *A. corrugatus*, and *A. corrugatus* × *A. konjac*, could develop two or more leaves, especially those of *A. konjac* × *A. corrugatus*, which developed as many as four leaves. This is unusual because normally one year old konjac has only one leaf. The weight of the corms of hybrids varied with hybrid combinations. The weight of corms of the one year old F1's are in the order *A. konjac* × *A. konjac* > *A. konjac* × *A. albus* > *A. albus* × *A. konjac*. The characters of F_1 is matroclinous. There exist certain morphological similarities among the different combinations, and a few plants have excellent characters. Rapid multiplication of them is currently under way in our study.

4. Summary and Discussion

It is considerably difficult in konjac breeding to cross *A. konjac* with other species. This is because (1) its life cycle is long and it normally flowers after 4 – 5 years from either seed or rhizomes; (2) there are different flower periods of the species; and (3) there are also some incompatibilities between them.

Through low – temperature – induction, the start of the flower period of *A. konjac* corms with flower buds can be delayed by 2 – 4 months. Treatment with GA_3 of the corms without flower buds can induce an excellent male sterile line and make it possible to cross with other late – flowering species. It is not useful to store the pollen of *A. konjac* at low temperature (– 18℃) and dry conditions. By directly pollinating with pollen of *A. albus* and excision of its stigma and then pollinated with *A. corrugatus*, to some degree, interspecific cross incompatibility can be over – come. However, by the above mentioned methods, crosses with *A. paeoniifolius* are unsuccessful. This show that they have greater incompatibility. This can be seen by their different number of chromosomes, *A. paeoniifolius* (2n = 28), *A. konjac* (2n = 26) (Long *et al*, 1989), This may cause the abnormal chromosome behavior during the gamete forming process.

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